ABSTRACT OF THE INVENTION

A high-sensitivity, low-background immuno-amplification assay is provided, which offers a streamlined workflow suitable for high-throughput assays of clinically relevant samples, such as blood and other bodily fluids. The assay comprises the use of two proximity members that each comprise an analyte-specific binding component conjugated to an oligonucleotide. Binding an analyte brings the oligonucleotide moieties of the proximity members in sufficiently close contact that the oligonucleotides form an amplicon. The presence of the analyte then is detected through amplification of the amplicon and detection of the amplified nucleic acids. The sensitivity of the assay of the present invention is improved by preventing spurious or non-specific amplicon formation by proximity members that are not complexed with an analyte. In one embodiment, target-independent amplicon formation is prevented by using hybridization blocker oligonucleotides that bind oligonucleotide moieties that are not hybridized to each other. Background is further reduced by providing a solid phase capture oligonucleotide that prevents amplicon formation until the captured complex is released.